A PREPARATIVE COLUMN FOR THE ION EXCHANGE ISOLATION OF AMINO COMPOUNDS

INTRODUCTION

The classical methods of MOORE AND STEIN[1] and the automatic modification of SPACKMAN, STEIN AND MOORE[2] for the resolution, identification and quantitative analysis of amino acids in protein hydrolysates have been major contributions to protein chemistry. The application of these methods to natural products, however, has been handicapped by the problem of ascertaining the purity of individual peaks, of identifying unknown compounds, and isolation of sufficient materials for study. Since these analytical ion-exchange methods are micro in scope, other more involved and more tedious procedures must be used to isolate these unknown materials for identification.

HIRS, MOORE AND STEIN[3] reported the use of a 150 × 1.8 cm column for the separation of the mixture resulting from the trypsin hydrolysis of oxidized ribonuclease. This resulted in only a 4-fold increase in capacity and they gave no indication of the comparative resolution obtained with the analytical and preparative columns. PARTRIDGE AND BRIMLEY[4] used three to four coupled columns made from glass pipe for a primary fractionation of the amino acids from protein hydrolysates. Subsequent separations of each fraction were made using new columns, resins and eluants.

This paper reports the results of scaling up the MOORE AND STEIN 100 cm column to the extent of 24 times. By use of this column, sufficient materials for analysis and preparation of derivatives can be obtained with a minimum of effort and change in the ordinary analytical method.

APPARATUS AND OPERATION

Fig. 1 is a schematic drawing of the scaled-up column. The dimensions were calculated by increasing the cross-sectional area by a factor of 24. The resulting column diameter was 44 mm I.D. and the cross-sectional area was 15.2 cm^2. This compares with the 9 mm I.D. analytical column with a cross-sectional area of 0.64 cm^2 or a factor of 23.92.

* Eastern Utilization Research and Development Division, Agricultural Research Service, United States Department of Agriculture, Philadelphia 18, Pa.

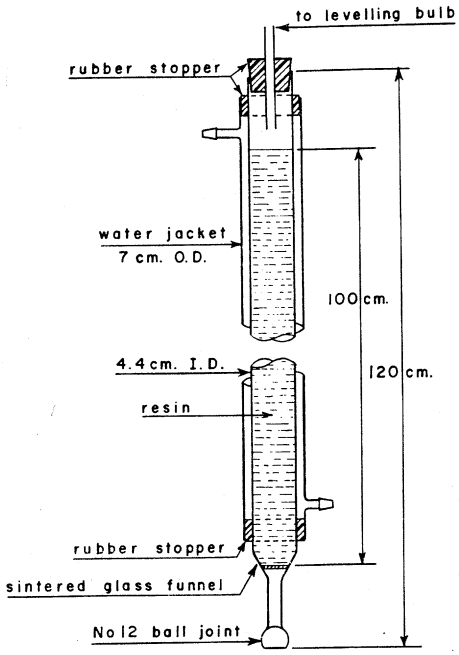

Fig. 1. The scaled-up column.

The resin employed was Dowex-50 X 8*, labeled minus 400 mesh. This preparation was wet sieved through a 200 mesh (U.S.) screen and the fines were removed by backwashing.

The column was constructed by sealing a 60 ml pyrex fritted glass (coarse) funnel to the end of a 115 cm tube, 44 mm I.D. After sealing a No. 12 ball joint to the funnel outlet[5], the overall length of the column was approximately 120 cm. The tube was fitted with rubber stoppers and a surrounding glass tube to jacket the column and control the column temperature.

After mounting the column in an upright position and checking the alignment with a plumb bob, it was filled with resin suspended in pH 3.42 buffer. The resin had been prepared for use according to the treatment of MOORE AND STEIN[1]. About 10–13 cm of buffer was placed in the tube after closing off the ball joint. The resin suspension was added down the side of the tube, by means of a funnel with a bent tip, until about 10–13 cm of column had been poured. The first was allowed to settle until the resin had formed a bed. The tubulation was opened and the buffer allowed to flow through the resin by gravity until no more resin remained in suspension and 10–15 cm of clear buffer remained above the resin bed surface. If the resin surface does not appear to be perfectly flat, the alignment of the column should be checked. Subsequent increments of 10–15 cm of resin suspension were added in this way until the column was about half filled. The remaining portion of the column was poured through a funnel with a

* Mention of a specific product does not specify recommendation of that product by the Department of Agriculture over any other equal product not mentioned.

U-shaped bend at the bottom to prevent disturbance of the surface of the previously poured and settled layer. This procedure was repeated until a packed column length of 100 cm was obtained. *Extreme care in pouring these columns is required to obtain maximum resolution.*

A 100 × 0.9 cm analytical column was prepared according to the method of MOORE AND STEIN using the same resin preparation as used for the preparative column.

Both columns, mounted on "Technicon" drop-counting fraction collectors, were run at the rate of 8 fractions per hour[5]; fractions of 24 ml and 1 ml were collected at the same flow rate (ml/min/ml resin) from the preparative and analytical columns, respectively. 1 ml aliquots were removed from each fraction from the preparative

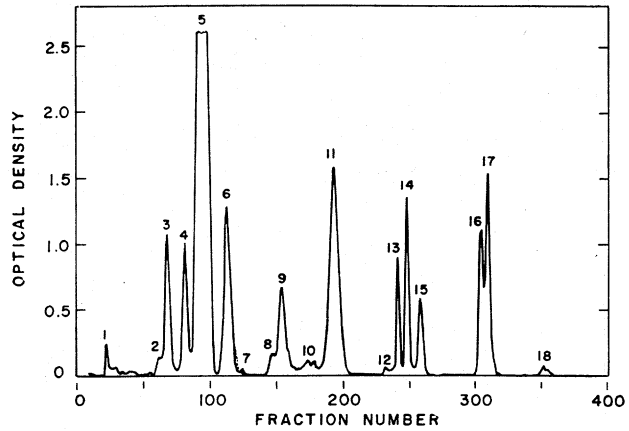

Fig. 2. Preparative column run.

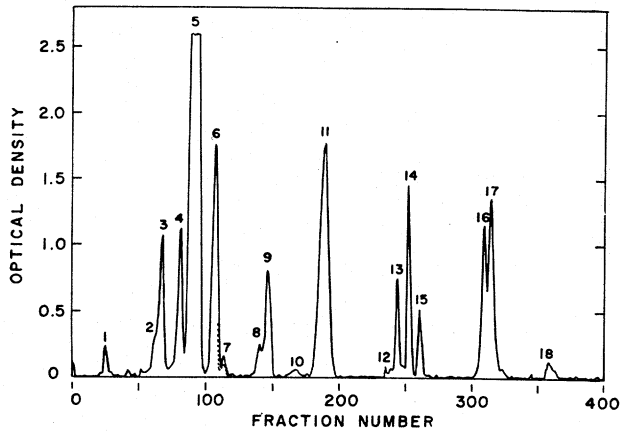

Fig. 3. Analytical column run.

The buffer change (3.42 pH to 4.25 pH) and the first temperature change (37.5° to 50°) were made as fraction 195 filled. The second temperature change (50° to 75°) was made 90 fractions later. Identification of peaks: 1, unknown; 2, unknown; 3, aspartic acid; 4, threonine; 5, asparagine, glutamine and serine; 6, glutamic acid; 7, proline (read at 440 mμ); 8, glycine; 9, α-alanine; 10, unknown; 11, valine; 12, unknown; 13, methionine; 14, isoleucine; 15, leucine; 16, tyrosine; 17, phenylalanine; and 18, β-alanine.

column and placed in the matched test tubes used in the regular analytical procedure. The ninhydrin color was developed and the optical densities were measured in a Beckman Model B spectrophotometer. Using samples of potato extract, in the ratio of 1 to 24 in volume, the results shown in Figs. 2 and 3 were obtained by use of the preparative and the analytical columns respectively. In practice, the remaining 23 ml of each fraction in the individual peaks were combined, desalted by ion exchange, concentrated and crystallized for use in X-ray diffraction, derivatization and analysis.

RESULTS AND DISCUSSION

The Figs. 2 and 3 show that the resolution obtained with the preparative column was as good or better than that obtained with the analytical column. (The slight variation in effluent volumes may be due in part to variation in fraction size. Similar results were obtained with columns 15 cm long for the basic amino acids.)

In special applications, where certain zones are separated relatively far from other materials, as much as 18 g of amino acid mixture have been resolved on the column. For materials closer together in their elution volumes, lesser amounts must be employed.

ACKNOWLEDGEMENT

The authors sincerely appreciate the help of FAIRIE LYN CARTER and THOMAS J. FITZPATRICK in building and using these columns.

SUMMARY

A preparative column is described, which gave a resolution equal to that of an analytical column run under the same conditions.

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